Elemental Fingerprinting of Bivalve Shells Using Laser Ablation ICP-MS to Evaluate the Dynamics of Larval Exchange

Lisa A. Levin
Integrative Oceanography Division
Scripps Institution of Oceanography
La Jolla, California 92093-0218
phone: (858-534-3579) fax: (858-822-0562) email: llevin@ucsd.edu

Grant Number: N00014-01-1-0473
http://levin.ucsd.edu/

LONG-TERM GOALS

The goal of this grant was the acquisition of equipment and development of techniques to apply trace element fingerprinting to the estimation of bay-ocean, bay-bay, and within-bay larval exchange rates in mussels of southern California. Ultimately we hope to compare realized dispersal patterns based on elemental fingerprinting of larval and recruit shells to those predicted from physical transport models. The focus of the request was purchase of a UP213 nm laser ablation unit for use with a double focusing, single collector, magnetic sector ICP-MS (Inductively Coupled Plasma-Mass Spectrometer).

OBJECTIVES

The primary project objective is to acquire instrumentation and develop protocols for measurement of trace elements in shells of larval and newly recruited mussels. Specific goals involve achieving high instrument sensitivity (low detection limits), precision and accuracy as well as small spatial resolution (i.e. 10-20 μm) so that larval trajectories can be reconstructed. The instrumentation will be used to assess the influence of mussel species, shell size, shell zone, development site and settlement location on trace elemental composition. For southern California mussel populations we hope to determine whether bay-released larvae develop inside or outside a specific bay, whether populations are self-seeding, and whether there is larval exchange with other bays or coastal populations.

APPROACH

A New Wave UP 213 nm Laser Ablation unit was recently purchased for use with a newly acquired magnetic sector ICP MS. Additional purchases include an integrated video capture device, and etower computer with monitor to run the Merchantek EO software that guides and manipulates the laser. To help integrate the hardware of the LA system and the ICP-MS, we also purchased an additional torch and an extra set of cones for the ICP-MS. Glitter software was bought for analysis of transient signals produced by laser ablation so that that the ICP software could turn them into concentrations.

Current efforts focus on protocol development and include mounting methods, assessment of appropriate standards for analyses, laser settings for ablating 500-2000 μm shells, communication between ablation and mass spec instrumentation, and application of programs to process raw elemental data. Initial efforts are aimed at *Mytilus* spp. shells. SEM is used to visualize shell ablation.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **30 SEP 2001**	2. REPORT TYPE	3. DATES COVERED **00-00-2001 to 00-00-2001**
4. TITLE AND SUBTITLE **Elemental Fingerprinting of Bivalve Shells Using Laser Ablation ICP-MS to Evaluate the Dynamics of Larval Exchange**		5a. CONTRACT NUMBER
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Integrative Oceanography Division,,Scripps Institution of Oceanography,,La Jolla,,CA,92093**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release; distribution unlimited**		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**5**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

WORK COMPLETED

Discussions with other laser ablation users and instrument demonstration led to the purchase of an New Wave Research Laser Ablation system Model UP 213.Al with a polarizer-analyzer, Dynamic Auto Focus. Since receiving this new tool, we have concentrated on protocol development. Optimal laser parameters such as ablation energy, duration, diameter and spacing were experimentally determined on sample material. We have considered options for external standards, and will be using commercially available glass disks (National Institute for Standards and Technology RPM 612, 614, and 616) for this purpose. Rare calcium isotopes will function as internal standards. In addition, we have been working to configure the ICP-MS hardware and software to communicate with the laser ablation system

Working with very small (<2mm) shells leads to a number of analytical hurdles. We have successfully determined how to (1) mount and section in order to be able to access and sample critical parts of the shell, (2) balance the time needed for analysis with the number of isotopes that can be used given limited shell material and (3) determine which isotopes provide the most consistent and relevant data for our purposes. Solution-based analyses have been used for this goal, as well as to calibrate the results acquired with liquid and solid analyses.

Until recently, solids needed to be dissolved in order to be analyzed using a mass spectrometer. By using a laser to examine the solid directly, it is possible to sample very minute (10-20 µm) parts of the shell, and therefore access and compare parts of the shell that correspond to different periods of the animal's development. Using this technology, we can not only distinguish the larval shell from the adult shell, but also analyze different parts of the larval shell (Fig. 1). This spatial resolution translates to enhanced temporal resolution; parts of the larval shell correspond to different development times of the larva. This type of information was previously impossible to obtain using solution-based methods.

Figure 1 depicts 16µm holes from a transect along the shell of a newly recruited mussel (900 µm total length). The small end of the shell contains the larval protoconch (137 µm x 240 µm, Fig. 1B), retained at settlement. The remainder of the shell, the dissoconch, was laid down after recruitment.

Information about larval origins and trajectories is essential for accurate modeling of population dynamics and species with planktonic larvae, and ultimately for effective management of coastal resources. Marine ecologists have long been concerned with the role of pre-recruitment processes and early life history, although they have been limited by their ability to track larvae, due to small size, low concentration, and relatively long planktonic periods. By further developing techniques to evaluate larval origins and exchange, this research will advance understanding of marine populations. A further understanding of bay to bay larval exchange should have a wide range of applications including assessment of the interdependence of different habitats, evaluation of controls on population dynamics, and assessment of pollution consequences.

TRANSITIONS

This award has allowed transition from ICP-OES solution-based to LA-ICP-MS solid phase analyses, opening vast opportunities for studying invertebrate shells as recorders of their environmental history.

RELATED PROJECTS

This project is related to the parent program “Application of elemental fingerprinting to evaluate the dynamics of larval exchange”. Collaborators include John Largier and Claudio Di Bacco. We are also working closely with Scripps graduate student Bonnie Becker, who is studying the influence of the Point Loma kelp forest on scales of dispersal and recruitment in mussels at the Cabrillo National Monument, San Diego, and with graduate student Joel Fodrie, who is using trace elemental fingerprinting to reconstruct utilization of southern California estuaries by juvenile halibut. The laser ablation unit comprises an important piece of equipment for their research. Conservation biologist Enric Sala will be examining elemental composition of otoliths (ear bones) in Gulf of California leopard grouper, to evaluate exchange among populations. Petrologists at Scripps will use the laser ablation system to analyze elements in order to partition them between magmatic melt and crystallizing solid. Elemental analyses of natural glasses and phenocrysts in lava will deliver information about magma history and sources.

PUBLICATIONS

None yet.

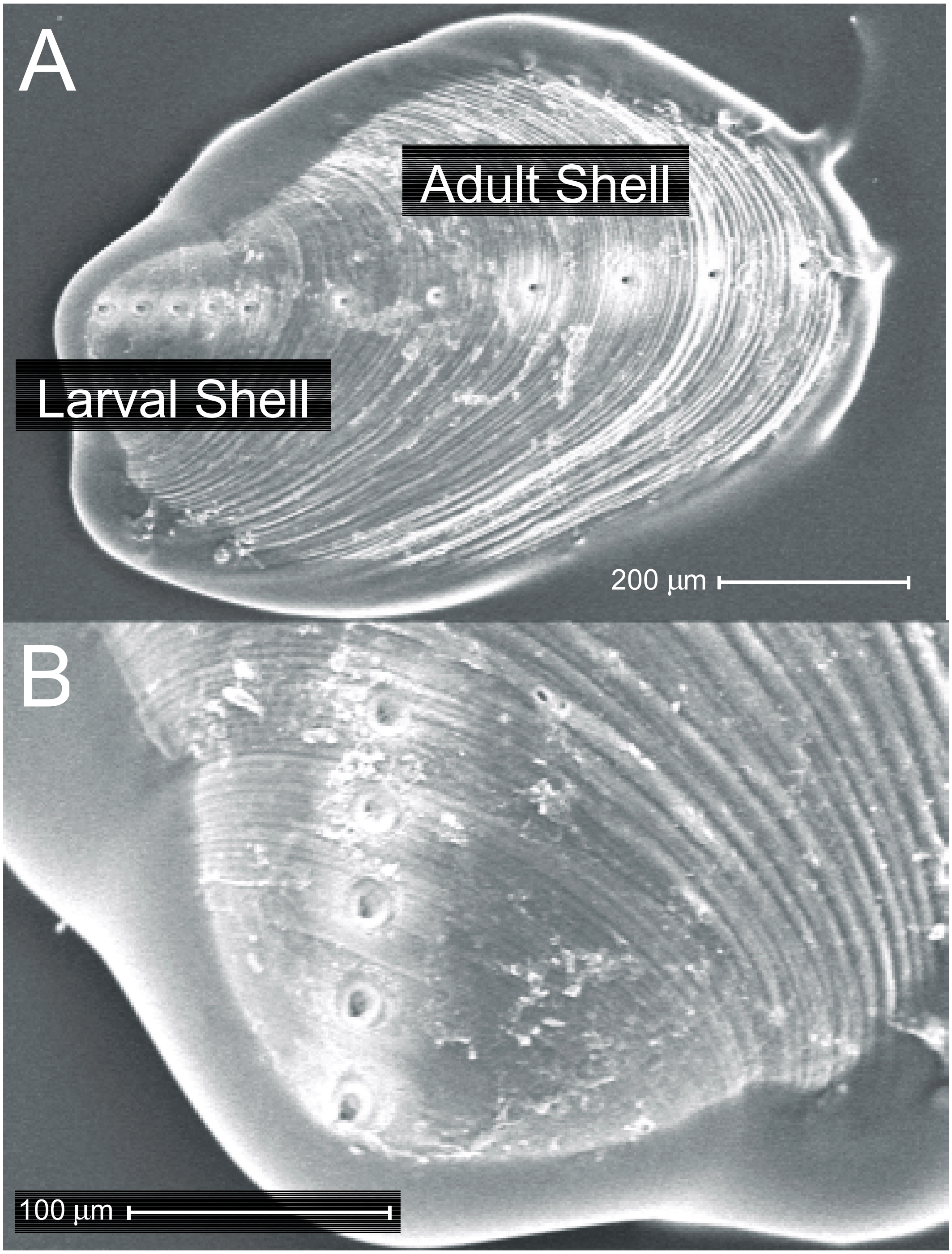

Figure 1. Scanning electron micrograph depicting 16-µm diameter holes made by a laser ablation transect across the shell of a newly recruited mussel from rocks near Scripps Institution of Oceanography, LaJolla, CA. (A) whole shell (B) close up view of larval shell. Ablated material is analyzed by Inductively Coupled Plasma Mass Spectrometry to obtain trace element signatures (fingerprints) of larval and post-settlement (recruit) portions of the shell. This information will be used to assess sites of larval development and exchange among populations.